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Distinct Strategies to Make Nucleosomal DNA Accessible

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Summary

One hallmark of ATP-dependent remodeling complexes is the ability to make nucleosomal DNA accessible to regulatory factors. We have compared two prominent human ATP-dependent remodelers, BRG1 from the SWI/SNF family and SNF2h from the ISWI family, for their abilities to make a spectrum of nucleosomal sites accessible. By measuring rates of remodeling at seven different sites on a mononucleosome and at six different sites on the central nucleosome of a trinucleosome, we have found that BRG1 opens centrally located sites more than an order of magnitude better than SNF2h. We provide evidence that this capability of BRG1 is caused by its ability to create DNA loops on the surface of a nucleosome, even when that nucleosome is constrained by adjacent nucleosomes. This specialized ability to make central sites accessible should allow SWI/SNF family complexes to facilitate binding of nuclear factors in chromatin environments where adjacent nucleosomes might otherwise constrain mobility.

Introduction

Most nuclear factors have limited access to eukaryotic DNA that is packaged into chromatin. ATP-dependent chromatin remodeling complexes can modulate the accessibility of DNA sequences in chromatin; therefore, they play a pivotal role in regulating nuclear events, such as transcription, replication, recombination, and repair (Becker and Horz, 2002; Fyodorov and Kadonaga, 2001). ATP-dependent remodelers have been suggested to expose nucleosomal DNA by sliding away of the histone octamer (Becker and Horz, 2002). Sliding provides an efficient way to alter and specify nucleosome positions; nonetheless, it does not appear to be an efficient way to open up multiple DNA sites across stretches of consecutive nucleosomes because of the constraints on sliding placed by adjacent nucleosomes. This raises the question of whether some ATP-dependent remodelers use strategies other than sliding to expose DNA.

The SWI/SNF and ISWI families are two of the best-characterized classes of ATP-dependent chromatin remodeling complexes (Becker and Horz, 2002; Narlikar et al., 2002). The two classes have different biological functions and display different biochemical activities. BRG1 and SNF2h represent the motor proteins of the major human SWI/SNF and ISWI family complexes, respectively. Both proteins alone have the basic biochemical activities of the whole complexes. The other subunits of these remodeling complexes are involved in targeting these remodeling activities in vivo and in enhancing the specific activities of the motor proteins.

In vivo studies have suggested that SWI/SNF and ISWI complexes have different biological functions. Studies from yeast, *Drosophila*, and mammals indicate that both these families are involved in transcription regulation but they appear to regulate different sets of genes (Armstrong et al., 2002; Chi et al., 2002; Fazzio et al., 2001; Holstege et al., 1998; Liu et al., 2001; Santoro et al., 2002; Yasui et al., 2002). SWI/SNF complexes have been found to participate in cell cycle regulation, and components of SWI/SNF complexes behave as tumor suppressors (Klochendler-Yeivin et al., 2002). ISWI-based complexes have been suggested to modulate higher order structure of the male X chromosome in *Drosophila* and to facilitate the binding of the cohesin complex to DNA sequences containing Alu sequences in cultured human cells (Deuring et al., 2000; Hakimi et al., 2002). Additionally, complexes containing apparent ISWI homologs have been suggested to function in the replication of heterochromatin and transcription repression (Bozhenok et al., 2002; Collins et al., 2002; Fazzio et al., 2001; Santoro et al., 2002; Strohner et al., 2001).

The two families of remodelers have distinct yet overlapping biochemical activities. For example, both alter the translational position of nucleosomes and increase DNA access (Boyer et al., 2000; Hamiche et al., 1999; Langst et al., 1999; Whitehouse et al., 1999). However, SWI/SNF and BRG1 can introduce changes in superhelicity in a closed circular nucleosomal array, but SNF2h cannot (Aalfs et al., 2001; Kwon et al., 1994). On the other hand, whereas ACF, an ISWI family complex, can assemble nucleosomes from histones and DNA, this activity has not been demonstrated for SWI/SNF family members (Ito et al., 1997). Also, ISWI family complexes can create regularly spaced nucleosomes, but SWI/SNF complexes have not been shown to have this activity (Corona et al., 1999; Ito et al., 1997; Varga-Weisz et al., 1997).

These two families also have different substrate specificities (Becker and Horz, 2002; Narlikar et al., 2002): histone N-terminal tails are important for the activities of the ISWI family complexes but are dispensable for SWI/SNF function. Both naked DNA and nucleosomal DNA stimulate the ATPase activity of SWI/SNF equally well; however, nucleosomal DNA preferentially stimulates the ATPase activities of ISWI-based complexes. Additionally, SWI/SNF binds to a core mononucleosome with little flanking DNA, while flanking DNA is important for the binding of the *Drosophila* ISWI protein to mononucleosomes.

One question raised by the different biochemical and

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biological activities of the two remodeler classes is whether they have different impacts on nucleosomes to accomplish their divergent in vivo roles. Here, we have addressed this question by comparing the effects of BRG1 and SNF2h on mono- and trinucleosomal templates and by characterizing some of the fundamental differences in their products. The results suggest that these remodelers use very different strategies to create DNA accessibility: SNF2h makes DNA accessible primarily by sliding away the histone octamer as proposed previously (Hamiche et al., 1999; Kassabov et al., 2002; Langst et al., 1999). In contrast, BRG1 can make DNA accessible by generating stable DNA loops without the requirement for sliding away the histone octamer. We propose that these fundamentally different ways of opening up DNA may facilitate two distinct components of chromatin remodeling in vivo: efficient modulation of nucleosome positions and exposure of DNA in nucleosomal regions that are locally constrained by adjacent nucleosomes.

Results

To compare how BRG1 and SNF2h alter DNA accessibility on a nucleosome, we first characterized their effects on a 202 bp mononucleosome substrate that can be efficiently remodeled by both BRG1 and SNF2h. We characterized the products created by these remodelers using native gel electrophoresis and then compared the rates at which a spectrum of nucleosomal sites was made accessible. Rate experiments were then extended to trinucleosome templates to examine whether the DNA ends on a mononucleosome had an impact on accessibility of sites. The purified BRG1 and SNF2h proteins used in this study (Figure 1A) had similar specific activities for remodeling (8 U/mg for BRG1 and 20 U/mg for SNF2h; see Experimental Procedures).

BRG1 and SNF2h Generate Different Remodeled Products from Mononucleosomal Substrates

A change in DNA accessibility on a mononucleosome can result from an alteration in histone octamer position within a mononucleosome, which can be detected using native polyacrylamide gel electrophoresis. Prior to remodeling, the histone octamers adopted different positions on the 202 bp DNA fragment, as suggested by bands of different mobilities on a native gel. The positions of the histone octamer in each band were mapped using standard procedures (Figure 1B) (Hamiche et al., 1999; Langst and Becker, 2001a). Remodeling by BRG1 and SNF2h altered the pattern of nucleosome mobility on a native gel (Figure 1C). The predominant BRG1 products were fast-migrating nucleosome species, while the predominant SNF2h products were slow-migrating species (Figure 1C, compare lanes 4 and 6; lanes 10 and 12; lane 16 and 18). Additionally, the major products generated did not change regardless of the initial starting nucleosome translational position (Figure 1C, compare lanes 4, 10, and 16; lanes 6, 12, and 18). We observed ATP-independent effects of both BRG1 and SNF2h on certain substrates (Figure 1C, compare lanes 1, 3, and 5; lanes 7, 9, and 11). These effects were different from the ATP-dependent effects and might have resulted from the ability of both proteins to bind to nucleosomes in the absence of ATP (Aalfs et al., 2001; Narlikar et al., 2001). BRG1- and SNF2h-remodeled products also displayed different gel mobilities when we used a mononucleosome substrate assembled onto DNA containing the *Xenopus* 5S rDNA sequence instead of the Shrader and Crothers nucleosome phasing sequence used here (data not shown). Together these results suggest that BRG1 and SNF2h generate different remodeled products.

A Larger Distribution of DNA Sites Is Accessible to MNase in BRG1 Products Than in SNF2h Products

To characterize the differences between the SNF2h and BRG1 products, we first used micrococcal nuclease (MNase) to probe the structures of these remodeled products. MNase, which preferentially cleaves DNA that is not in contact with a histone octamer, was added to the reaction mixture after terminating the remodeling reactions using excess ADP followed by competitor DNA (see Experimental Procedures). The amount of DNA added was sufficient to compete away the remodeling proteins as determined by native gel analysis (data not shown). Similar moles of DNA fragments ($>$90% recovery of starting material) were resistant to MNase cleavage following SNF2h and BRG1 remodeling. This indicated similar product recoveries and ruled out significant loss of histones in both remodeling reactions. Following MNase digestion and deproteinization, greater than 90% of the SNF2h products were resolved as a band of 150 $\pm$ 10 bp, indicating that SNF2h-remodeled products had $\sim$150 bp of DNA occluded by a histone octamer (Figure 2A). In contrast, BRG1 products displayed unexpected heterogeneity in the lengths of MNase-resistant DNA ranging from 60 to 202 bp (Figure 2A).

Mapping the major MNase-resistant DNA species (150 $\pm$ 10 bp) of the SNF2h products revealed that the predominant products were centrally located nucleosomes (Figure 2B), as expected from the mobility of the SNF2h products on the native gel (Figures 1B and 1C). These results are consistent with previous observations that remodeling by the ISWI protein mainly results in nucleosomes located around one predominant translational position, though the predominant histone octamer location may vary depending on the identity of the DNA templates used (Langst et al., 1999).

All the MNase-resistant DNA fragments generated from the BRG1 products (60–202 bp) were mapped. The region containing DNA ranging from 60 to 202 bp was divided into ten parts; DNA was eluted from each gel slice and individually mapped (Figure 2C; see also Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/11/5/1311/DC1). Fragments smaller than 150 $\pm$ 10 bp always contained one of the two DNA ends, suggesting that they did not arise simply from overdigestion by MNase near the entry and exit points of nucleosomal DNA.

The wide array of MNase-resistant DNA fragments in the BRG1 products must reflect different regions of DNA that are protected by histone octamers. One interpretation of this data is that the BRG1 products contain mononucleosomes in different translational positions (Figure 2C). If this is the case, the BRG1 products should resolve

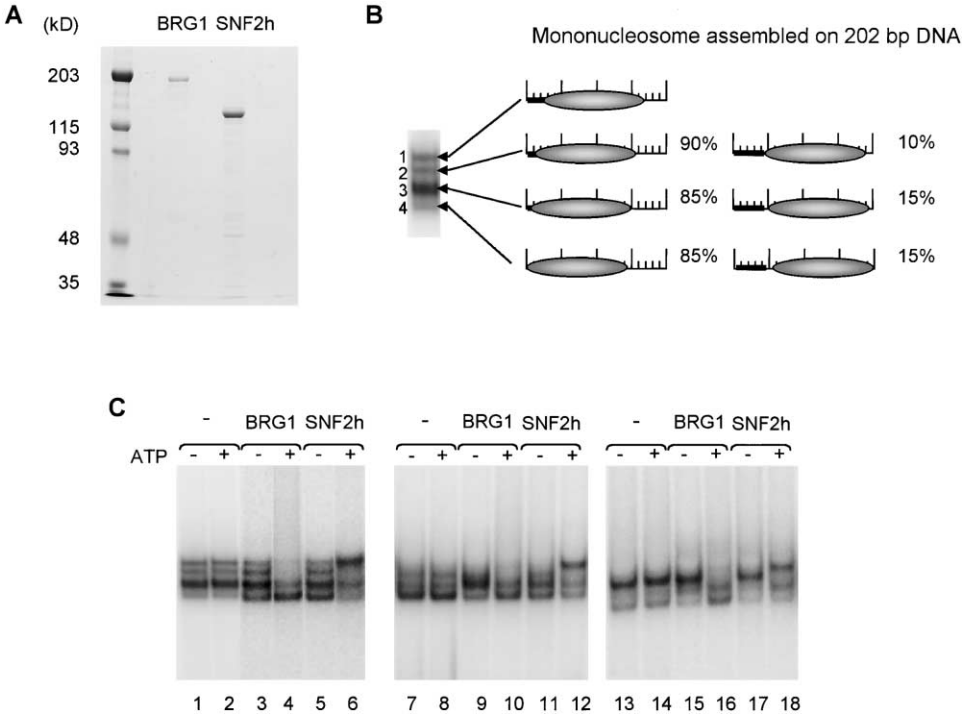

Figure 1. BRG1 and SNF2h Generate Different Remodeled Products

(A) Coomassie blue staining of BRG1 and SNF2h resolved by 8% SDS-PAGE.

(B) Mononucleosomes, assembled on a 202 bp, ^{32}P-body-labeled DNA fragment, were resolved as four bands on a 5% native polyacrylamide gel. The histone octamer positions in each band were mapped according to standard procedure and are indicated as ovals (Hamiche et al., 1999; Langst and Becker, 2001a). The solid box represents a 40 bp GT nucleosome phasing sequence (Schnitzler et al., 1998). Distance between ticks is 10 bp.

(C) Three different glycerol gradient nucleosome fractions were used in independent remodeling reactions (see the Experimental Procedures).

as multiple bands on a native gel. However, most BRG1-remodeled products migrate at a single position on a native gel (Figure 1C, lanes 4, 10, and 16). Hence, we could not interpret the MNase data simply in terms of histone octamer positions of canonical nucleosomes. This raised the possibility that the distinct MNase sensitivity pattern observed in BRG1 products reflected the presence of DNA exposed on the surface of the histone octamer, possibly via loop formation (Figure 7). Some BRG1 products could also be dinucleosome-like species observed previously with core mononucleosomes (Lorch et al., 1998; Schnitzler et al., 1998), although we detected less than 2% of such a species on a native gel under the reaction conditions used here. Nevertheless, the significant differences in the MNase sensitivity of the products created by SNF2h and BRG1 led to the prediction that SNF2h would make DNA accessible in a limited region (e.g., end positions, see Figure 2B), whereas BRG1 would make DNA accessible throughout the 202 bp DNA template.

BRG1 Is Better at Opening Up Internal Nucleosomal Sites Than SNF2h

To test this prediction, we used restriction enzyme accessibility assays to monitor the rates of DNA exposure at different positions on the 202 bp mononucleosome. Restriction enzyme sensitivity is a stringent and quantitative assay for DNA accessibility and thus can be used to determine the rate of a remodeling reaction (Logie and Peterson, 1997; Narlikar et al., 2001; Polach and Widom, 1995). We created seven DNA templates that were identical except for the location of the PstI site and measured the rate constants for BRG1- and SNF2h-catalyzed exposure of each PstI site (Figure 2D).

These two proteins demonstrated strikingly different, ATP-dependent site-exposure rate profiles (Figure 2D). For SNF2h-catalyzed remodeling, the rate constants of PstI site exposure at different positions varied by 100-fold. Sites at position 46 and 50 were exposed efficiently while every site that was within the central 90 bp was exposed inefficiently. These results are consistent with a histone octamer-protected area after position 50 as suggested by the mapping data in Figure 2B. In contrast, for BRG1-catalyzed remodeling, the rate constants of PstI site exposure at different positions varied by at most 3-fold, demonstrating that the location of a PstI site had very little effect on the ability of BRG1 to render the site accessible.

It was possible that the differences in exposure rates arose from bound SNF2h differentially occluding the DNA sites. To control for this, we measured site exposure by SNF2h under conditions for which the exposure rate of any given restriction site increases linearly over a 10-fold difference in SNF2h concentration. These conditions ensure that SNF2h is subsaturating so that most of the nucleosomes are not bound by SNF2h. Under

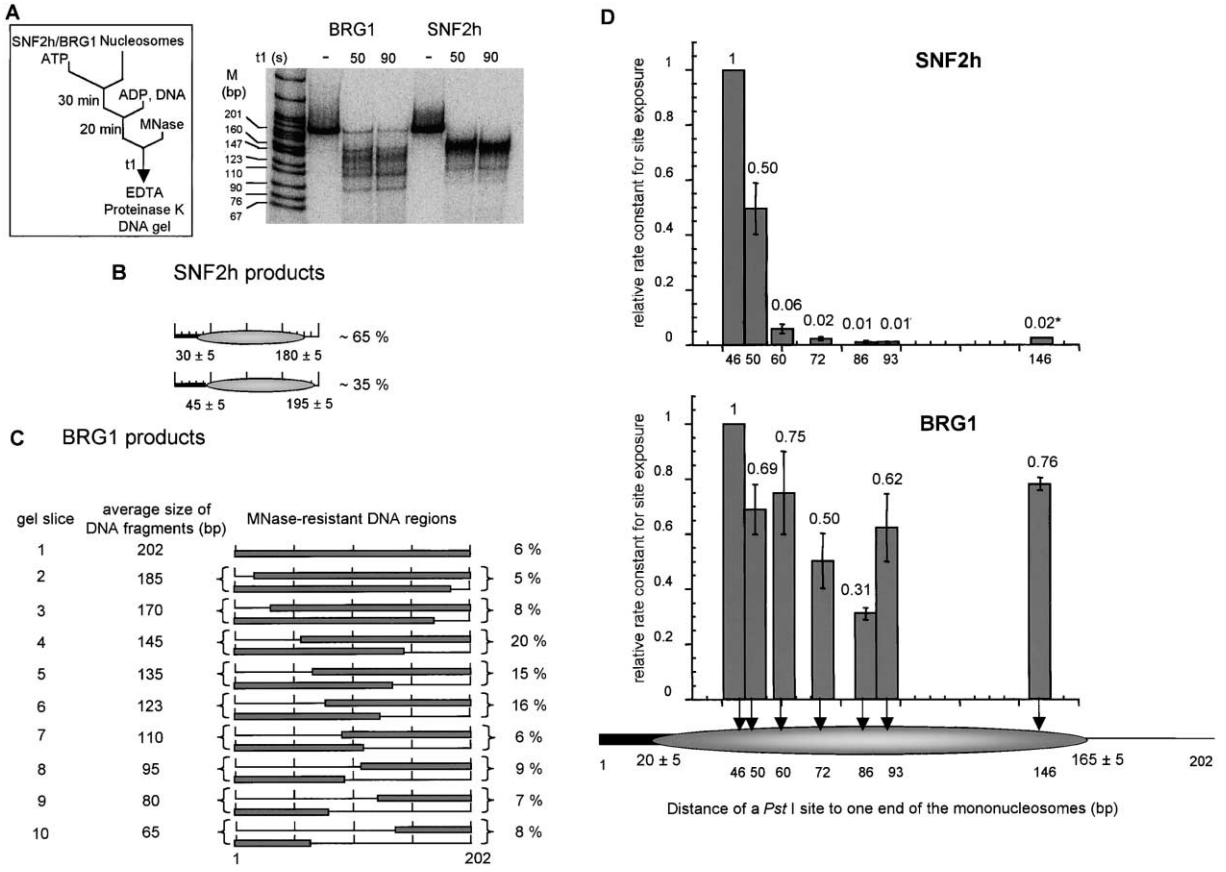

Figure 2. Mapping of the BRG1- and SNF2h-Remodeled Products

(A) BRG1- and SNF2h-remodeled products were treated with MNase, deproteinized, and resolved on an 8% polyacrylamide gel.

(B) Mapping the major SNF2h products. Ovals represent the nucleosomal region.

(C) Mapping BRG1-remodeled mononucleosomes. Bars represent DNA regions protected by the histone octamer from MNase digestion (see text). DNA fragments ranging from the average sizes (as shown) ±10 bp were mapped. DNA fragments with an average size of 65, 95, 170, or 185 bp are more spread out and thus are less visible in (A).

(D) Mononucleosome remodeling profiles of SNF2h and BRG1 monitored by continuous restriction enzyme accessibility assays. Mononucleosomes were heated at 55°C for 2 hr before assays, so that ~70% of all mononucleosomes had histone octamers localized between position 20 ± 5 and 165 ± 5, as determined by MNase and restriction enzyme mapping (data not shown) (Narlikar et al., 2001). The rate constants for cutting each PstI position were normalized to that of position 46 for BRG1 (0.2–0.3 min^{-1}) and SNF2h (0.2–0.4 min^{-1}): the normalized values are shown above the bars. At positions 46 and 50, SNF2h increased the rate of PstI exposure by at least 30-fold relative to reactions without ATP. BRG1 increased the rate of PstI exposure at all positions by at least 30-fold relative to reactions without ATP. (*No increase in DNA exposure relative to the reaction with no ATP.)

these conditions, the relative rate constants for opening of the different DNA sites did not change for any given SNF2h concentration, demonstrating that the differences in exposure rates do not arise from differentially bound SNF2h (data not shown).

Adjacent Nucleosomes Create Barriers for Remodeling by SNF2h but Not for BRG1 or Human SWI/SNF

The comparisons above were performed with mononucleosomes, which by definition have DNA ends immediately adjacent to the nucleosome. In the simplest case, the DNA ends are expected to act as barriers for histone octamer movement. However, it is possible that DNA ends might have also contributed to some of the significant differences in the abilities of SNF2h and BRG1 to expose nucleosomal DNA. Indeed, it has been recently proposed that the DNA ends in a mononucleosome participate in the generation of stable DNA loops after SWI/SNF remodeling (Kassabov et al., 2003). To control for this and other consequences of DNA ends, we generated a trinucleosomal template, which ensures that the central nucleosome is not flanked by DNA ends but instead by nucleosomes. The central nucleosome has limited space to slide due to flanking nucleosomes. If flanking nucleosomes create the same type of barrier to sliding as DNA ends, then remodeling by SNF2h and BRG1 should generate similar patterns of restriction enzyme accessibility on the central nucleosome as were seen on the mononucleosome: SNF2h should more efficiently open up sites near the entry and exit points of the central nucleosome than sites close to the dyad, and BRG1 should be able to open up all sites with similar efficiencies (Figure 3).

We assembled trinucleosomes on six 509 bp DNA fragments and separated the trinucleosomes from na-

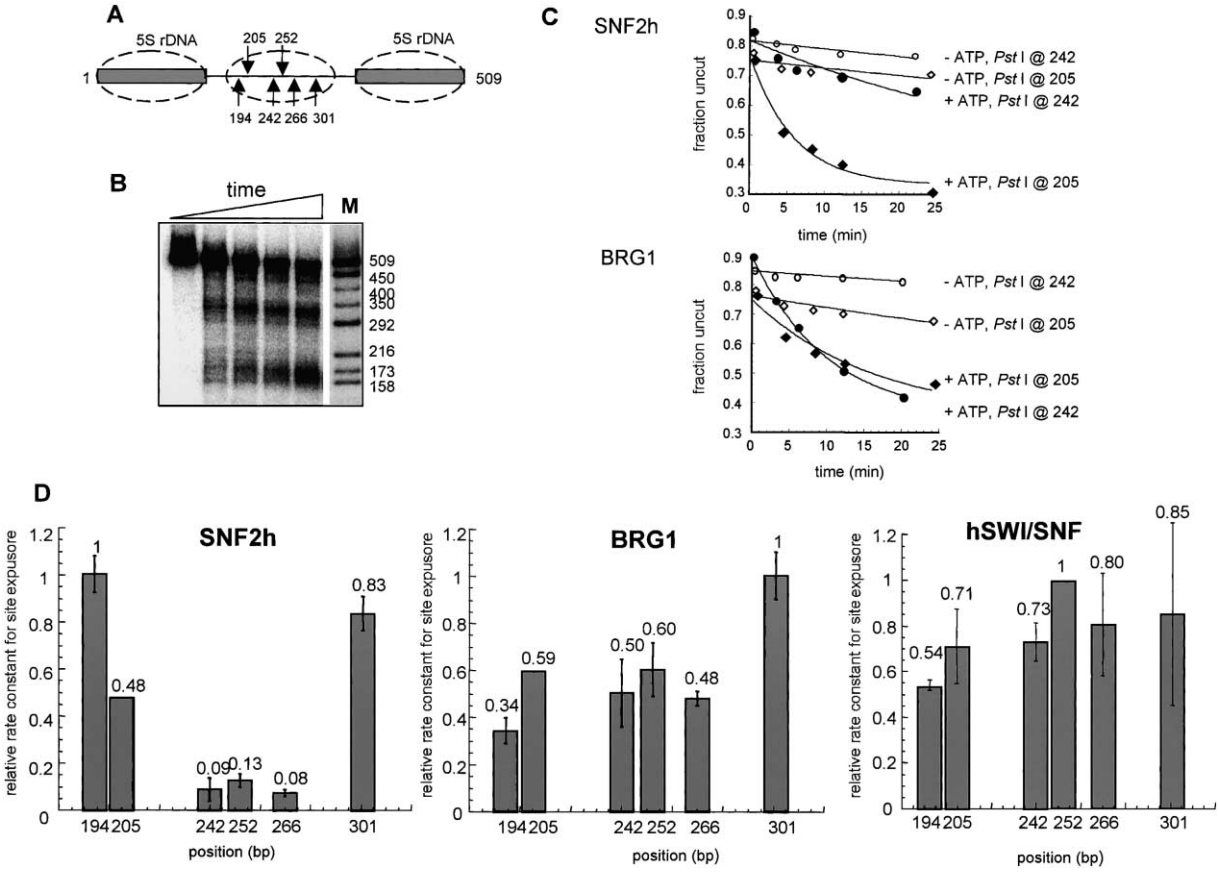

Figure 3. Trinucleosome Remodeling Profiles of SNF2h and BRG1 Monitored by Restriction Enzyme Accessibility Assays

(A) A schematic illustration of the 509 bp DNA templates used to assemble trinucleosomes. The PstI sites in the different templates are indicated by arrows. Heating trinucleosomes at 55°C for 2 hr before assays ensured that greater than 75% of trinucleosomes had the central 160 bp region occluded by histone octamers, as judged by restriction enzyme accessibility (data not shown).

(B) A representative trinucleosomal template was treated with MNase for varying times and deproteinized, and the products were resolved on a 5% polyacrylamide gel.

(C) Examples of remodeling reactions monitored by restriction enzyme accessibility on trinucleosome templates containing a PstI site at position 205 or 242. Reactions have PstI continuously present.

(D) The rate constants for opening each PstI position were measured relative to that for opening up position 205 for SNF2h (0.1–0.3 min^{-1}) and BRG1 (0.1–0.2 min^{-1}), and position 252 for hSWI/SNF; the relative values were then normalized with respect to the highest remodeling rate constants (positions 194, 301, and 252; for SNF2h, BRG1 and hSWI/SNF, respectively). Normalized values are shown above the bars. SNF2h opened up positions 194, 205, and 301 at least 30-fold faster than reactions without ATP. BRG1 and hSWI/SNF opened up the different positions at least 10-fold faster than reactions without ATP.

ked DNA and partially assembled mono- and dinucleosomes on a glycerol gradient (Experimental Procedures). All of them contained a 5S rDNA nucleosome positioning sequence (Ura et al., 1995) on each end and were identical except for the location of the PstI site (Figure 3A). The PstI site was introduced at a different position within the central 150 bp of each DNA template (Figure 3A). Fully assembled trinucleosomes were distinguished from partially assembled nucleosomal species based on their mobility in a glycerol gradient and native gel as well as by partial MNase digestion (Figure 3B and Supplemental Figure S3 at http://www.molecule.org/cgi/content/full/11/5/1311/DC1). SNF2h and BRG1 were allowed to remodel these trinucleosomes in the presence of PstI. Reactions were terminated at various times, and samples were then deproteinized and assayed on a DNA gel to determine the rate constant of exposing each PstI site. Some representative examples are shown in Figure 3C.

SNF2h demonstrated a gradient in rate constants for site exposure and opened the positions near the entry and exit points of the middle nucleosome ~12-fold more efficiently than the central positions (Figure 3D). In contrast, BRG1 exposed all the PstI sites with similar rate constants (Figure 3D) and in a manner that did not correlate with the distance of the site from the entry and exit points. The human SWI/SNF complex purified from HeLa cells also opened up these different sites with similar rate constants, confirming that the behavior of BRG1 reflected a fundamental property of the SWI/SNF complex. Dissociation of the histone octamers from the trinucleosomes was not responsible for the different remodeling profiles of BRG1 and SNF2h based on analysis of the trinucleosomal templates on a native gel before and after remodeling (data not shown). These results mirror the results obtained with the 202 bp mononucleosome (Figure 2D).

SNF2h family members have been suggested to ex-

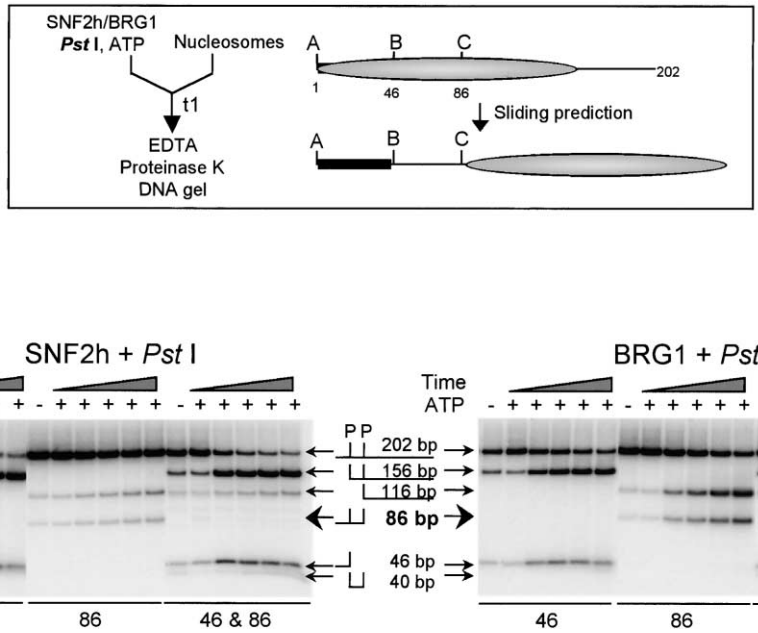

Figure 4. Analyzing Accessibility of Adjacent DNA Sites to Test a Sliding Model

Three mononucleosomes were used in this assay; one had two PstI sites located at both position 46 and 86, and the others had a single PstI site at either position 46 or 86 (controls). We used gradient fractions that were enriched for nucleosomes containing the end-positioned histone octamers (band #3, Figure 1B). To quantify the amount of the 86 bp fragment that was further digested at position 46, we first normalized the amount of the 86 bp fragment to the amount of the 116 bp fragment. The 116 bp fragment is also generated from a cut at position 86 but remains unaltered by a cut at position 46. We then compared how this normalized amount changed in the presence of an additional PstI site at position 46. At the last time point of 25 min, only 7% of the 86 bp fragment remained in the SNF2h reaction with nucleosomes having PstI sites at 46 and 86. In contrast, in the corresponding reaction for BRG1, 50% of the 86 bp fragment remained after 25 min. The −ATP lane shows a 25 min time point.

pose nucleosomal DNA by sliding the histone octamer with respect to the DNA (Hamiche et al., 1999; Kassabov et al., 2002; Langst et al., 1999). The above results are consistent with this model as SNF2h more efficiently opens up sites that can be made accessible by sliding away the histone octamers (i.e., sites near the edge of a nucleosome) than sites that are likely to be occluded in a canonical nucleosome containing ~145 bp of DNA that is constrained in movement by surrounding nucleosomes or ends of DNA (i.e., sites near the center of a nucleosome). It is possible that BRG1 creates wider access to restriction enzymes by using the same mechanism as does SNF2h, but slides the histone octamers to a greater extent than does SNF2h. On the templates used here, such extensive sliding would result in nucleosomes containing less than 145 bp of DNA. Alternatively, BRG1 might be able to expose DNA by making DNA loops within the bounds of the histone octamer (Figure 7, see Discussion). Comparison of rates of opening of two adjacent sites on the same nucleosome can help distinguish between these possibilities.

Analyzing Accessibility at Two DNA Sites Simultaneously on a Mononucleosome

If a given DNA site (site C in Figure 4) is exposed by sliding the histone octamer away from this site, then every time this site becomes exposed, all sites that lie closer to the initial nucleosome position (e.g., site B in Figure 4) will also become exposed. We constructed DNA templates that had PstI sites at positions 46, 86, or both 46 and 86. Both of these positions are occluded by the histone octamer in the majority of the starting nucleosome population (Figure 1). For the mononucleosomes with two PstI sites, the 116 and 86 bp bands represent the fraction of PstI-accessible nucleosomes at position 86 only. If any of this nucleosome population is also accessible to PstI at position 46, then the 86 bp fragment will be further digested to form two fragments of size 46 and 40 bp. Greater than 93% of the SNF2h-remodeled products that were PstI accessible at position 86 were also PstI accessible at position 46, since less than 7% of the intact 86 bp fragment was detected. This result is consistent with the model that SNF2h exposes DNA by sliding away histone octamers. In contrast, 50% of the 86 bp DNA fragment generated by BRG1 remained undigested, indicating that 50% of the BRG1 products that were PstI accessible at position 86 were PstI inaccessible at position 46. This result is inconsistent with the simplest prediction for nucleosome sliding but does not exclude the possibility that BRG1 exposes site C by sliding the histone octamer toward site A. Further analysis of DNA access in BRG1-remodeled products at three sites simultaneously ruled out this possibility (see Supplemental Figure S2 at http://www.molecule.org/cgi/content/full/11/5/1311/DC1). Together, these results suggest that BRG1 can expose a DNA site without sliding the histone octamer away from this site.

BRG1 Can Create DNA Access within the Bounds of the Histone Octamer on the Central Nucleosome of a Trinucleosome

To further investigate whether BRG1 could expose a DNA site without sliding the histone octamer away from this site, we directly tested whether BRG1 could generate DNA loops within the histone bounds. To avoid possible artifacts from DNA ends, we focused on the centrally localized nucleosomes within the trinucleosomes.

A subset of centrally located nucleosomes can be released by using RsaI and SacI. We measured the accessibility of the unique NsiI site present within this subset of central nucleosomes. We cleaved the BRG1 and SNF2h products with RsaI and SacI to release the 200 bp fragment containing nucleosomes located between these two sites. If this central nucleosome has a canonical nucleosome structure, then the NsiI site should always be occluded wherever the histone octamer is located on the 200 bp RsaI-SacI DNA fragment. In contrast, if the NsiI site is exposed within the histone bounds in a noncanonical structure, then this site should show increased accessibility in the excised remodeled products relative to the nonremodeled substrate.

Cutting of excised 200 bp central nucleosome by NsiI generates 109 and 91 bp fragments. We could not quantify the amount of NsiI cutting by quantifying these fragments as these fragments can also be generated without simultaneous exposure of the RsaI and SacI sites. Hence, we quantified NsiI digestion by measuring the decrease in the amount of the 200 bp fragment relative to the amount of the 150 and 159 bp 5S fragments also generated by the RsaI and SacI cuts. These 5S fragments served as an internal standard as they do not contain an NsiI site.

The central nucleosomes excised from the BRG1 products showed a ~3-fold higher NsiI accessibility relative to the −ATP background (Figure 5B). The human SWI/SNF complex also showed a comparable increase in NsiI accessibility relative to the −ATP background. In contrast, the central nucleosomes excised from the SNF2h products did not show increased NsiI accessibility above background (Figure 5B). NsiI accessibility in the −ATP background probably comes from the small fraction of templates that are nonnucleosomal in the region between RsaI and SacI (see below).

It was formally possible that remodeling by BRG1 made the central region nucleosome-free and gave the above results. However, a native gel analysis of the RsaI- and SacI-cut nucleosomes revealed that BRG1 did not dissociate histone octamers from DNA in the central region significantly in an ATP-dependent manner (the fraction of total template that was nonnucleosomal in the central region was 0.020 ± 0.012 [−ATP] and 0.027 ± 0.013 [+ATP] for BRG1, and 0.030 ± 0.018 [−ATP] and 0.031 ± 0.015 [+ATP] for SNF2h; see also the Experimental Procedures).

These data demonstrate that BRG1, in contrast to SNF2h, can create noncanonical nucleosomes that have DNA stably exposed within the histone bounds of a nucleosome. Together with the results of Figures 2D and 3, the data also suggest that the disparities in SNF2h and BRG1 behavior reflect fundamental differences in how the two enzymes make sites accessible and are not the consequence of any particular template.

SNF2h Cannot Remodel BRG1 Products

The above results revealed important differences in the abilities of BRG1 and SNF2h to create DNA access: BRG1 can create nucleosomes with DNA exposed on the histone surface (noncanonical nucleosomes), while SNF2h creates canonical nucleosomes with altered histone octamer positions. To further confirm that BRG1 creates noncanonical nucleosomes, we determined whether SNF2h could remodel BRG1 products using mononucleosomes.

We used a two-step protocol in which BRG1 remodeling could occur in the first step and SNF2h remodeling could occur during the second step (Figures 6A and 6B). To monitor DNA access of the remodeled products, PstI was added at the end of step two after terminating the reactions with ADP and competing the remodelers away from mononucleosomes with excess DNA (see the Experimental Procedures). In the second step, the reaction was diluted 5-fold while adding excess SNF2h over BRG1. If BRG1 creates DNA access at a central position within a mononucleosome by sliding the histone octamer away from this site, SNF2h should be able to reset BRG1-remodeled nucleosomes to occlude this centrally located position as it does with other nucleosomal substrates (Figures 1C and 2B). To test this, we looked at the accessibility of position 93; position 93 was used because BRG1 exposes position 93 whereas SNF2h moves a nucleosome over it (Figures 2B and 2D).

Upon reaction with BRG1, ~30% of the nucleosomes became PstI accessible at position 93 (Figure 6A, reaction 3). Most of the accessible PstI sites were on mononucleosomes and not naked DNA because less than 6% naked DNA was detected by native gel analysis (data not shown). As expected, after addition of SNF2h to regular nucleosomes, PstI access at position 93 did not increase above background (Figure 6A, reaction 2), reflecting the presence of a histone octamer over the site in the SNF2h products (Figure 2B). In contrast, after addition of SNF2h to the BRG1 products, ~80% (~25/30%) of the PstI-accessible BRG1 products remained PstI accessible, indicating that SNF2h could not reposition the BRG1-remodeled nucleosomes to occlude position 93 as it does in the case of other nucleosomal substrates (reaction 4 in Figure 6, and Figures 1C and 2D).

It was possible that the above result was due to residual BRG1 activity that kept generating PstI accessibility in SNF2h-remodeled nucleosomes. However, a control reaction showed that after incubation at 30°C for 30 min in step 1 and the 5-fold dilution in step 2, BRG1 had undetectable remodeling activity (reaction 5, Figure 6A, see the Experimental Procedures). Another explanation for the above result was that BRG1 prevented access to SNF2h in step 2 by remaining bound to the nucleosomes. To test this possibility, we used nucleosomes with a PstI site at position 50 as this site can be exposed by SNF2h. We incubated BRG1 with these nucleosomes in the absence of ATP in step 1. Previous work has shown that BRG1 binds mononucleosomes with similar affinities in the absence and presence of ATP (Narlikar et al., 2001). The reaction was then diluted 5-fold while adding ATP and excess SNF2h over BRG1 in step 2 (Figure 6C). Any detectable activity has to be due to SNF2h because BRG1 had undetectable activity in step 2 (Figure 6A, reaction 5). Under these conditions, SNF2h

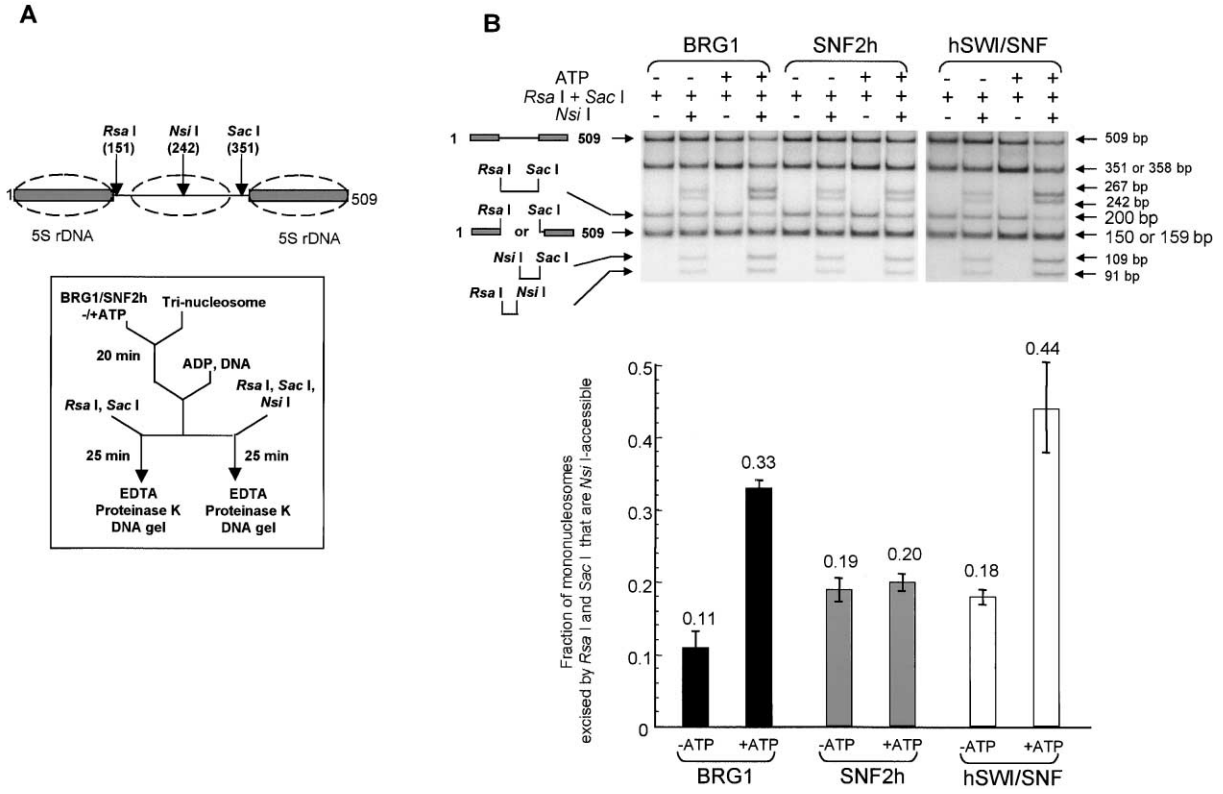

Figure 5. Detection of DNA Access within the Bounds of Histone Octamers in BRG1- and SWI/SNF-Remodeled Trinucleosomes

(A) A schematic illustration of the 509 bp trinucleosomal template. RsaI and SacI were used to excise the central 200 bp mononucleosomes from remodeled trinucleosomes, and NsiI was used to determine the fraction of this mononucleosome population that is NsiI accessible.

(B) The amount of 200 bp DNA fragment in each reaction was normalized with respect to the total amount of the 151 and 159 bp DNA fragments; these will not change upon addition of NsiI (see text). We determined the fraction of NsiI-accessible 200 bp mononucleosomes by comparing the decrease in the normalized amounts of 200 bp fragments between doubly and triply digested samples. The background cutting is most likely from naked DNA in the samples that are generated in an ATP-independent manner (see text).

made position 50 accessible at similar rates whether step 1 contained just buffer or BRG1, indicating that SNF2h had access to nucleosomes under the reaction conditions of Figure 6A (Figure 6C).

The above inability of SNF2h to remodel BRG1 products contrasts with the experiments of Figure 1C that demonstrated that a variety of starting nucleosome positions formed following salt gradient dialysis could all be remodeled by SNF2h. These data are also in contrast to the ability of CHRAC, an ISWI-containing complex, to remodel nucleosomes that have previously been remodeled by ISWI (Langst and Becker, 2001a). Together, these data suggest that most of the BRG1 products (~80%) that have exposed DNA in the central region of the mononucleosome are not good substrates for SNF2h and thus may not be canonical nucleosomes.

Discussion

The results presented here provide a biochemical basis for the different biological functions of the ISWI and SWI/SNF family of complexes. We demonstrate that these two classes of ATP-dependent remodelers have dramatically different abilities to open up a spectrum of nucleosomal sites and that the differences are not simply due to differences in translational position of the remodeled histone octamer. SNF2h appears to generate a limited set of altered nucleosomal positions that are most easily explained by sliding of the histone octamer with respect to the DNA, supporting previous results with other ISWI family members (Hamiche et al., 1999; Kassabov et al., 2002; Langst et al., 1999). In contrast, BRG1 appears to generate a wide distribution of nucleosomal products that cannot all be easily explained by sliding of the histone octamer to give canonical nucleosomes. SNF2h cannot reset several of the altered nucleosome products generated by BRG1, further suggesting that some of the BRG1 products are not canonical nucleosomes. Finally, our data strongly suggest that a significant fraction of the nucleosomes remodeled by BRG1 and by the SWI/SNF complex contain DNA loops that are stably exposed within the bounds of the histone octamer. We cannot detect this type of nucleosomes among SNF2h-remodeled products. Thus, ATP-dependent remodeling of a nucleosome can have strikingly different outcomes depending upon which class of remodelers is active.

Robust regulation of genome structure requires not only ways to efficiently alter and specify nucleosome position but also ways to efficiently expose DNA sequences despite constraints imposed by surrounding

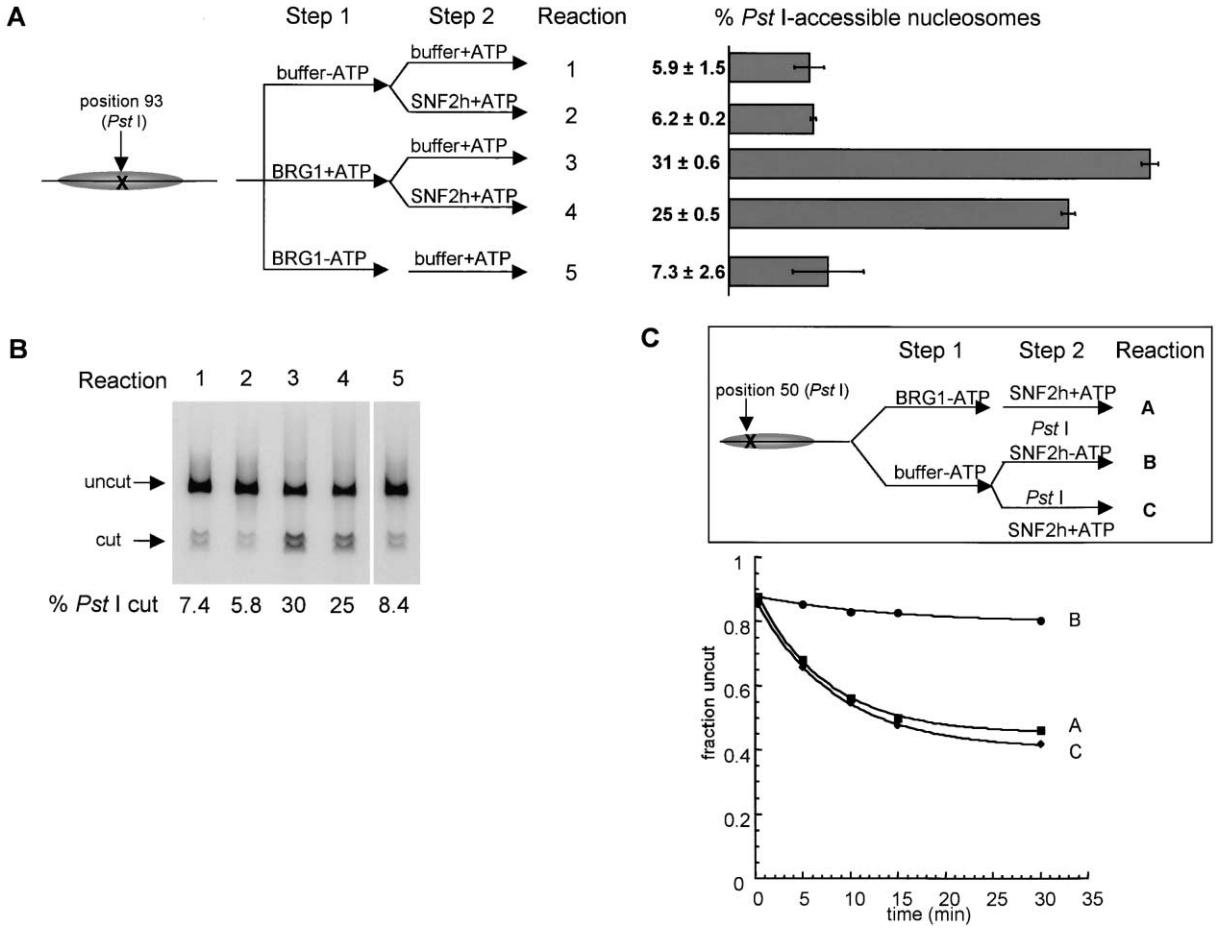

Figure 6. SNF2h Could Not Remodel All BRG1-Remodeled Products

(A) Mononucleosomes with a PstI site located at position 93 were subjected to two-step reactions as outlined. Five nanomolars of BRG1 (or buffer) was used to remodel mononucleosomes for 30 min at 30°C (Step 1). The reactions were then diluted 5-fold, and SNF2h (or buffer) was added at 10 nM (Step 2). After another incubation at 30°C for 30 min, ADP and DNA were added to terminate the reactions. Stopped reactions were digested with PstI (2 U/μl) for 20 min at 30°C to determine the fractions of PstI-accessible remodeled nucleosomes, then deproteinized, and resolved on polyacrylamide gels. Shown is the average PstI accessibility from three independent experiments with standard errors plotted.

(B) The DNA gel for one of the data sets used in (A).

(C) SNF2h can gain access to the nucleosome in the presence of BRG1. Mononucleosomes with a PstI site located at position 50 were used. Five nanomolars of BRG1 (or buffer) was mixed with mononucleosomes for 30 min at 30°C in the absence of ATP (Step 1). The reactions were then diluted 5-fold, and SNF2h was added at 10 nM with or without ATP at 30°C (Step 2) along with PstI to monitor whether SNF2h had continuous access to the nucleosomes. At various times, aliquots were deproteinized and assayed on gels. Reactions A and C have similar remodeling rate constants (0.12 and 0.13 min^{-1}, respectively), indicating that SNF2h can gain access to the mononucleosome substrates in our assay conditions regardless of the presence of BRG1.

features of the chromatin. Sliding the histone octamer with respect to the DNA is important for the appropriate spacing of nucleosomes following replication and for the efficient positioning of nucleosomes in regions that require explicit positioning. Nonetheless, exposure of nucleosomal DNA by sliding the histone octamer away from the DNA is constrained by the boundaries of surrounding nucleosomes as recapitulated by the trinucleosome template (Figure 3). Thus, maximal flexibility of DNA access in the nucleus requires, a priori, ways for exposing DNA other than simply sliding away of the histone octamer.

BRG1 and human SWI/SNF are able to create access to sites throughout the central nucleosome of the trinucleosome with similar efficiencies (Figure 3). We propose that this is a result of the ability of BRG1 and SWI/SNF to alter nucleosome conformation in a manner that will similarly expose sequences within the bounds of the histone octamer in vivo and that can therefore lead to versatile access to any nucleosomal sequence regardless of position. This ability provides an explanation for remodeling phenomena that are not easily explained by nucleosome sliding such as induction of the yeast *PHO5* promoter upon phosphate starvation, which involves generating access to a stretch of DNA organized into four adjacent nucleosomes (Becker and Horz, 2002).

Models for the Differential Effects of BRG1 and SNF2h on Nucleosomes

What could be the origins of the different products generated by BRG1 (alone or as part of hSWI/SNF) and SNF2h? The different products could reflect distinct

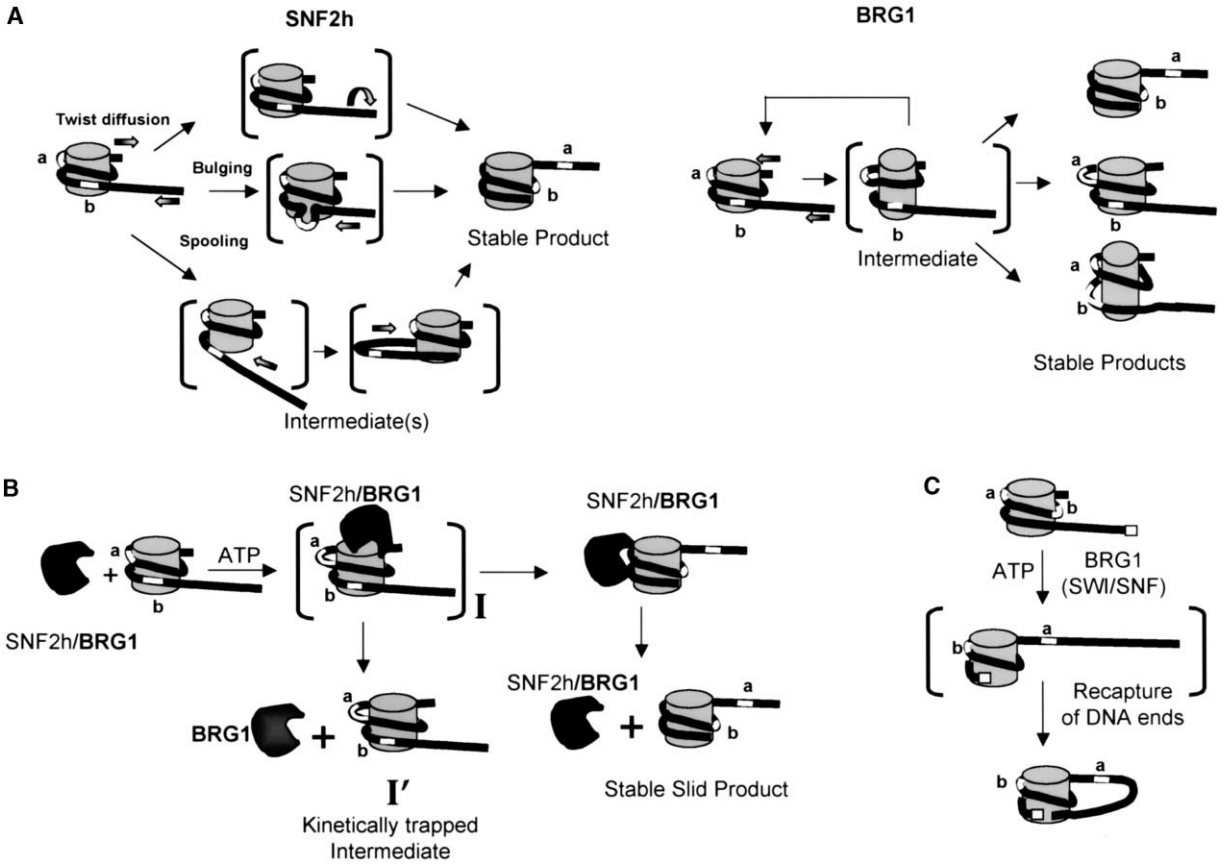

Figure 7. Models Accounting for Different Products Generated by SNF2h and BRG1

(A) SNF2h and BRG1 use different mechanisms to remodel nucleosomes. SNF2h may slide histone octamers via twist diffusion, bulging, or spooling to expose a DNA site such as (a). In contrast, BRG1 may create an altered nucleosome conformation; in this model, repositioning of a histone octamer is not a necessary outcome for exposure of site (a) or (b).

(B) BRG1 (and hSWI/SNF) and SNF2h remodel nucleosomes by the same mechanism, and the two reactions proceed though similar intermediates such as I. In this model, BRG1 and hSWI/SNF release I more often than SNF2h does and thus create a kinetically trapped intermediate (I′) with site (a) exposed within the histone bounds. The structures depicted for the intermediate and final products in (A) and (B) are hypothetical and could involve changes in the conformation of DNA, histones, or both.

(C) BRG1 and SWI/SNF may exposes site (a) by sliding the histone octamer off the DNA ends. The exposed DNA end subsequently rebinds the histone octamer to form a stable loop (Kassabov et al., 2003).

mechanisms as schematically depicted in Figure 7A. SNF2h may open up DNA primarily by sliding and via intermediates in which the DNA is twisted, spooled, or bulged (Langst and Becker, 2001b). BRG1 (or SWI/SNF) may create an intermediate with a different conformation of the DNA or histones. This would then collapse to stable structures, some of which contain DNA that is exposed within the bounds of the histone octamer and some of which contain altered histone positions (Figure 7A).

In an alternative model, both SNF2h and BRG1 slide the histone octamer to a different position via intermediates that contain DNA loops on the histone surface (Figure 7B). In the case of BRG1 and SWI/SNF, some fraction of these intermediates may dissociate from the enzyme before getting converted to the final product. In the absence of remodeling, these intermediates could be kinetically trapped and thus present with the final products. A simple prediction made by this model is that SNF2h can act on the dissociated intermediates (I′) and convert them to the final products. However, we observe that SNF2h cannot act on BRG1-remodeled nucleosomes (Figure 6), leading us to favor the model outlined in Figure 7A.

Another model (Figure 7C) that has been recently proposed to explain SWI/SNF remodeling entails sliding of the histone octamer in a mononucleosome beyond the ends of the DNA, followed by recapture of the DNA end to generate stable DNA loops (Kassabov et al., 2003). This model requires the presence of flanking DNA ends to explain stable loop formation and also predicts that a site near the entry and exit points of the DNA (Figure 7Ca) will be exposed faster than a site closer to the dyad (Figure 7Cb). However, we observe that (1) SWI/SNF can generate stable DNA loops in the central nucleosome of a trinucleosome, which is not flanked by DNA ends, and (2) SWI/SNF can expose sites near the dyad with similar or greater rates than sites near the entry

and exit points (Figures 2D and 3D). Hence, the results presented here are not consistent with this model (Figure 7C).

Experimental Procedures

Protein Purification

C-terminal tagged BRG1 and SNF2h were expressed in SF9 cells using a baculovirus overexpression system and purified using M2-affinity chromatography (Aalfs et al., 2001; Phelan et al., 1999). Human SWI/SNF, which was Flag-tagged at the Ini1 subunit, was purified from HeLa cells as described previously (Sif et al., 1998).

Nucleosome Assembly

Mono- and trinucleosomes were reconstituted with HeLa histone octamers using salt gradient dialysis (Luger et al., 1997). DNA fragments were generated using PCR and body-labeled with [^{32}P] α-dATP. Mononucleosomes were purified as described previously (Narlikar et al., 2001). Trinucleosomes were purified on a 5 ml 10%–45% glycerol gradient (35,000 rpm, 16 hr, 4°C), and the fractions were analyzed by native gel and MNase digestion (Figure 3B and Supplemental Figure S3 at http://www.molecule.org/cgi/content/full/11/5/1311/DC1). The gradient buffer contained 50 mM Tris HCl (pH 7.5), 100 mM KCl, and 1 mM EDTA.

Mapping Nucleosomal Substrates and Remodeled Products

Nucleosome positions of mononucleosomes and remodeled products were mapped as described previously (Hamiche et al., 1999; Langst and Becker, 2001a). In brief, nucleosome species were gel purified and subjected to limited MNase digestion. The major MNase-resistant DNA (150 $\pm$ 10 bp) was gel purified, and restriction enzymes were then used to map its identity. For BRG1 and SNF2h products, the remodeling reactions were terminated using ADP at 10 mM followed by excess naked DNA (100 ng/μl, kb ladder from NEB) to compete away any bound BRG1 or SNF2h. This reaction was subsequently subjected to limited MNase digestion. The major MNase resistant DNA fragments were treated as above to map their identities (see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/11/5/1311/DC1). Addition of ADP simultaneously with ATP at the beginning of the remodeling reaction prevented remodeling, confirming the effectiveness of using ADP to stop the reactions (data not shown).

ATP-Dependent Remodeling Assays: Measurement of Rate Constants

All remodeling reactions were performed in 12 mM HEPES (pH 7.9), 10 mM Tris HCl (pH 7.5), 60 mM KCl, 8% glycerol, 4 mM $MgCl_2$, 2 mM ATP Mg, and 0.02% NP40 at 30°C. Most reactions contained BRG1 (5–50 nM) and SNF2h (1–10 nM) in excess of nucleosomal substrates ($<$1 nM). Specific activities were determined using excess substrate over enzyme.

Both proteins can act catalytically under conditions of excess nucleosomes (see below) (Aalfs et al., 2001; Narlikar et al., 2001). We, however, used enzyme excess over substrate to drive the reaction to completion for two reasons. (1) This allowed us to use the entire reaction course to get accurate rate constants instead of using initial rates with substrate excess. This greatly reduces errors from the 5%–15% of background cutting that is seen in the absence of ATP as initial rates can only be measured for $<$15% of product formation. (2) For reactions in which we were comparing end products, this ensured that most of the nucleosomes were remodeled. The differences in the outcomes of BRG1 and SNF2h remodeling were maintained under conditions of saturating as well as subsaturating remodeler concentrations (data not shown).

Reactions for which rate constants were measured were initiated by addition of ^{32}P-labeled nucleosomes after preincubating the remaining components, including 0.4 U/μl PstI, together at 30°C for 5–10 min. Aliquots were removed at various times, quenched, deproteinized, and analyzed on polyacrylamide gels (Narlikar et al., 2001). Rate constants were determined by fitting the entire reaction (fraction of uncut substrate versus time) to first-order (exponential decay) fits.

In other reactions where end products were analyzed, PstI was used at 0.4–2.5 U/μl. Before resolving remodeled products on 5% polyacrylamide gels (0.5$\times$ TBE), ADP was added at 10 mM to terminate remodeling reactions followed by DNA at 100 ng/μl (1 kb DNA ladder from NEB) to compete away bound BRG1 or SNF2h.

Determining Specific Activities of BRG1 and SNF2h

The specific activities of BRG1 and SNF2h were determined under conditions of saturating and excess substrates (ATP and 202 bp mononucleosomes) over enzymes to ensure that we assayed all the active BRG1 or SNF2h. The reactions were performed at 30°C under the same buffer condition as above. One unit is defined as the amount of enzyme required to generate 1 pmole of PstI-accessible mononucleosomes (at position 50) per minute at 30°C. Rate constants were obtained from initial rates determined by linear fits to data for the first 10% of cut nucleosomes.

Estimating the Fraction of Trinucleosomes that Are Nonnucleosomal between RsaI and SacI

Nucleosomes were treated with RsaI and SacI according to the scheme in Figure 5A, except for the last step of deproteinization. The products were resolved on a 4% polyacrylamide, 0.5$\times$ TBE native gel. The fraction of trinucleosomes that were nonnucleosomal in the central 202 bp was obtained by quantifying the amount of 202 bp naked DNA relative to all the other nucleosomal species (tri-, di-, and mononucleosomes).

Investigating SNF2h Action on BRG1 Products

Five nanomolars of BRG1 was used in remodeling reactions (step 1). After 30 min, these reactions were diluted 5-fold; SNF2h was added at 10 nM and allowed to react for 30 min (step 2). Subsequently, ADP was added at 10 mM to terminate the reactions followed by competitor DNA at 100 ng/μl. Nucleosomes were further digested with PstI (2 U/μl) for 20 min at 30°C. PstI-digested samples were then deproteinized and resolved on a 6% gel to determine the fraction of PstI-accessible nucleosomes.

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